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# Integrated process for production of surfactin

## Part 2. Equilibrium and kinetic study of surfactin adsorption onto activated carbon

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### Abstract

Previous work has presented kinetics of pure surfactin adsorption onto activated carbon. Being an efficient biosurfactant, the lipopeptide surfactin has been produced in a bioprocess supported by the strain *Bacillus subtilis* ATCC 21332. This work is aimed at studying surfactin recovery directly from the culture medium. A thermodynamic study is carried out. Referring to adsorption capacity, the thermodynamic study confirmed that the adsorption of pure surfactin is an exothermic process. The capacity of surfactin adsorption from culture media shows that the activated carbon could be used as efficient adsorbent for surfactin recovery in an integrated process. The study shows the importance of the temperature for process control. Aimed at fixed bed column design, surfactin adsorption modelling on a single microporous pellet is demonstrated.

*Keywords:* Adsorption; Surfactin; Thermodynamics; Kinetics

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### 1. Introduction

Some strains of *Bacillus subtilis* produce a variety of lipopeptides. The pattern of lipopeptides produced is strain-dependant. The strain ATCC 21332 produces the surfactin lipopeptide [1], which is one of the most powerful biosurfactants known to act as detergent on biological membranes [2]. Surfactin has the advantages of biodegradability, low toxicity and biocompatibility. Because of its surface active properties and being environmentally friendly, the surfactin is of great industrial and commercial interest. Potential industrial applications include enhanced oil recovery, crude oil dribbling lubricants, surfactant aided bioremediation of water insoluble pollutants and uses in the health care and in food processing industries [3–7]. However, biosurfactants are not widely available because of their high production cost, resulting primarily from low strain productivities and high recovery expenses. The foregoing efforts

regarding improvement of biosurfactant's yield and purity motivated us to develop a novel process strategy involving process integration. Process integration is an attractive approach to foam elimination and to produce valuable products of high quality at reduced costs. Typical example is the integration of a fermentation process with liquid isolation of a specified reaction product [8]. In the present study, we focused on direct recovery of surfactin from supernatants collected at bioreactor exit following filtration.

The adsorption of surfactin on activated carbon is studied at batch conditions. The effects of parameters such as culture medium composition, adsorbent particle diameter and temperature were investigated. In the goal to design a fixed bed column, surfactin adsorption modelling on a single microporous pellet is presented.

### 2. Experimental

Like in our previous work [9], Merck activated carbon (Ref. 1.02514.1000) with spherical geometry was used. The surfactin

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## Nomenclature

|                    |   |
|--------------------|---|
| $A$                | constant of Temkin isotherm (mg/L)                                  |
| $b$                | constant of Temkin isotherm   |
| $B$                | constant of Dubinin isotherm  |
| $C_e$              | equilibrium liquid-phase surfactin concentration (mg/L)             |
| $C_p$              | heat capacity   |
| $C_t$              | liquid-phase surfactin concentration at time $t$ (mg/L)             |
| $E$                | constant of Dubinin isotherm  |
| $h$                | heat transfer coefficient   |
| $\Delta H^\circ$   | enthalpy of adsorption (kJ/kg)                                      |
| $k_a$              | kinetic constant of adsorption                                      |
| $k_d$              | kinetic constant of desorption                                      |
| $K_F$              | constant of Freundlich isotherm ((mg/g)/(mg/L) <sup>1/n</sup> )     |
| $K_L$              | constant of Langmuir isotherm (mg <sup>-1</sup> )                   |
| $N$                | number of experimental data   |
| $q_e$              | equilibrium solid-phase surfactin concentration (mg/g)              |
| $q_m$              | maximum adsorption capacity of adsorbent (mg/g)                     |
| $q_s$              | maximum adsorption capacity of adsorbent in Dubinin isotherm (mg/g) |
| $\bar{q}$          | average adsorption capacity of adsorbent (mg/g)                     |
| $R$                | universal gas constant (=8.314 J/(mol K))                           |
| $s$                | external surface area per unit particle volume                      |
| $\Delta S^\circ$   | entropy of adsorption (J/K kg)                                      |
| $t$                | time (h)  |
| $T$                | temperature (K)   |
| $1/n$              | heterogeneity factor  |
| <i>Geek letter</i> |   |
| $\Theta$           | fractional coverage   |

adsorption had been tested by using carbon of different particle-size, between 0.45 and 1.40 mm. Because the limitation step in the proposed integrated process is the fermentation rate, a simple column of fixed bed is envisaged to the surfactin separation and purification. Therefore, in further work, activated carbon with particle size of 1.40 mm and 0.9 was used with respect to potential application in fixed bed (the smaller the activated carbon particles, the more difficult it is to collect them).

In this work, the adsorption of pure surfactin from aqueous buffer solution has been studied and the surfactin was adsorbed directly from the culture media. The culture media with surfactin was obtained with the *B. subtilis* ATCC 21332 in the medium described by Leclere et al. [10]. The sorption capacity of the adsorbents has been studied using the adsorption isotherm technique. In each adsorption experiment, 20 mL culture media and 25 g/L activated carbon were placed in a 150 mL flask. Then, the flask was laid in a controlled environment incubator shaker (New Brunswick Scientific Co., USA) at 200 rpm. The surfactin

concentration was determined by reverse phase C18 HPLC [9]. Following the adsorption, the amount of surfactin was calculated by using a procedure described by Liu et al. [9].

## 3. Results and discussion

### 3.1. Adsorption of pure surfactin from aqueous solution

In our previous work, adsorption of pure surfactin onto activated carbon from aqueous buffer solution was studied. The isotherms for adsorption of surfactin onto the activated carbon at 20, 30 and 40 °C are shown in Fig. 1 [9]. It is shown that temperature has a pronounced effect on the adsorption capacity of the activated carbon. The increase of temperature accompanied by decrease of surfactin adsorption capacity suggests that the adsorption process of surfactin micelles onto the activated carbon is exothermic. The highest adsorption capacities of carbon for pure surfactin at 20, 30 and 40 °C are about 39, 28 and 18 mg/g carbon, respectively. Fig. 1 shows also that at lower surfactin concentrations the amount of surfactin adsorption at equilibrium,  $q_e$ , rises sharply and thereafter the increase is gradual with the increase of the solute concentration and  $q_e$  levels off.

Some isotherm models including Freundlich, Langmuir, Temkin and Dubinin-Radushkevich equations were used to fit the experimental data.

The Langmuir isotherm assumes monolayer adsorption onto a surface containing a finite number of adsorption sites with no transmigration of adsorbate in the plane surface. The Langmuir isotherm is given as

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \quad (1)$$

While plotting  $C_e/q_e$  against  $C_e$ , a straight line with slope  $1/q_m$  was obtained.

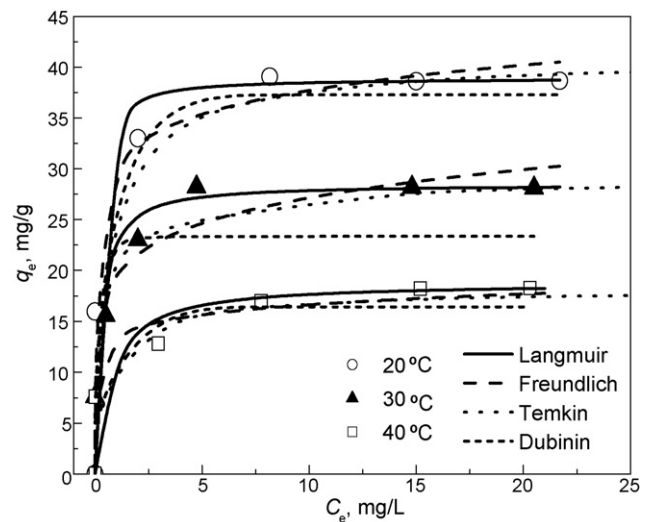


Fig. 1. Experimental results (symbols) and model correlation (lines) of isotherms of pure surfactin adsorption from aqueous solution onto activated carbon.

The Freundlich isotherm, on the other hand, assumes heterogeneous surface energies, in which the energy term in Langmuir equation is a function of surface coverage:

$$q_e = K_F C_e^{1/n} \quad (2)$$

Temkin and Pysher considered the effect of indirect adsorbate/adsorbate interactions on adsorption isotherm. The heat of adsorption of all molecules in the layer would decrease linearly with coverage due to adsorbate/adsorbate interactions. The Temkin isotherm has been used in the form as follows:

$$q_e = \left( \frac{RT}{b} \right) \ln(AC_e) \quad (3)$$

A plot of  $q_e$  versus  $\ln C_e$  allows to determine parameters  $b$  and  $A$ .

Another popular equation for the analysis of isotherms of a high degree of rectangularity is Dubinin-Radushkevich isotherm which is, as follows:

$$q_e = q_s \exp(-B\varepsilon^2) \quad (4)$$

where  $\varepsilon$  can be correlated:

$$\varepsilon = RT \ln \left( 1 + \frac{1}{C_e} \right) \quad (5)$$

The constant  $B$  gives the mean free energy  $E$  of sorption per molecule of the sorbate when it is transferred to the surface of the solid from infinity in the solution and can be computed by using the relationship:

$$E = \left[ \frac{1}{\sqrt{2B}} \right] \quad (6)$$

A plot of  $\ln q_e$  versus  $\varepsilon^2$  enables the constants  $q_s$  and  $E$  to be determined.

Isotherm parameters for the four isotherms at different temperatures are given in Table 1 along with the values of the correlation coefficients and the error functions values. Fig. 1 and Table 1 compare the different models proposed. It is seen that this adsorption is accomplished in two stages, i.e. a first stage of low concentration and a second one at high concentration. The transition between the two parts is close to 5 mg/L. We believe that the Langmuir model is more appropriate, since it is most important to represent the maximum adsorption capacity. Moreover, this model is suitable to use at different temperatures. The shape of adsorption isotherm is perhaps related to solid-liquid interaction (the progressive filling of adsorption sites) and/or to the CMC concentration. In the previous work [9], we showed that before the adsorption, the surfactine was demicellized then the surfactine was adsorbed. In the last step, because the concentration is higher than the CMC value, we obtain a surfactine micelle and this micelle may fill the active carbon pore.

### 3.2. Thermodynamics adsorption of pure surfactin from aqueous solution

The Langmuir model was originally developed to represent chemisorption on a set of distinct localized adsorption sites. The

Table 1  
Parameters corresponding to different isotherm equations

| $T$ ( $^{\circ}\text{C}$ )  | $q_m$ (mg/g)                    | $K_L$ (mg $^{-1}$ ) | $R^2$ |
|-----------------------------|---------------------------------|---------------------|-------|
| <b>Langmuir</b>             |                                 |                     |       |
| 20                          | 38.92                           | 8.16                | 0.999 |
| 30                          | 28.5                            | 4.24                | 0.999 |
| 40                          | 18.86                           | 1.48                | 0.996 |
| $T$ ( $^{\circ}\text{C}$ )  | $K_F$ [(mg/g)/(mg/L) $^{1/n}$ ] | $1/n$               | $R^2$ |
| <b>Freundlich</b>           |                                 |                     |       |
| 20                          | 30.49                           | 0.0922              | 0.991 |
| 30                          | 19.66                           | 0.1399              | 0.966 |
| 40                          | 13.6                            | 0.0878              | 0.947 |
| $T$ ( $^{\circ}\text{C}$ )  | $A$ (g $^{-1}$ )                | $b$                 | $R^2$ |
| <b>Temkin</b>               |                                 |                     |       |
| 20                          | $8.598 \times 10^5$             | $1.032 \times 10^3$ | 0.987 |
| 30                          | $1.693 \times 10^4$             | $1.131 \times 10^3$ | 0.898 |
| 40                          | $9.240 \times 10^5$             | $2.486 \times 10^3$ | 0.891 |
| $T$ ( $^{\circ}\text{C}$ )  | $q_s$ (mg/g)                    | $E$                 | $R^2$ |
| <b>Dubinin-Radushkevich</b> |                                 |                     |       |
| 20                          | 37.3                            | $1.294 \times 10^4$ | 0.968 |
| 30                          | 23.35                           | $1.157 \times 10^4$ | 0.827 |
| 40                          | 16.4                            | $1.447 \times 10^4$ | 0.850 |

basics assumptions on which the model is based areas follows:

1. Molecules are adsorbed at a fixed number of well-defined localized sites.
2. Each site can hold one adsorbate molecule.
3. All sites are energetically equivalent.
4. There is no interaction between molecules adsorbed on neighboring sites.

Considering the exchange of molecules between adsorbed and liquid phase:

$$\text{Rate of adsorption} : k_a C(1 - \Theta) \quad (7)$$

$$\text{Rate of desorption} : k_d \Theta \quad (8)$$

where  $q_s$  is the total number of sites per unit weight or volume of adsorbent and  $\Theta = q/q_s$  is the fractional coverage. At equilibrium, the rates of adsorption and desorption are equal:

$$\frac{\Theta}{1 - \Theta} = \frac{k_a}{k_d} C = K_L C \quad (9)$$

where  $K_L = k_a/k_d$  is the adsorption equilibrium constant. Eq. (9) can be rearranged to the commonly quoted form:

$$\Theta = \frac{q}{q_m} = \frac{K_L C}{1 + K_L C} \quad (10)$$

This expression shows the correct asymptotic behavior for monolayer adsorption since at saturation  $C \rightarrow \infty$ ,  $q \rightarrow q_s$ , and  $\Theta \rightarrow 1$ . While at low sorbate concentrations, Henry's law is

approached:

$$\lim_{q \rightarrow 0} \left( \frac{q}{c} \right) = K_L q_m = K' \quad (11)$$

$q_s$  is supposed to represent a fixed number of surface sites and it should therefore be a temperature-independent constant while the temperature dependence of the equilibrium constant should follow a Van't Hoff equation:

$$K_L = K_0 \exp \left( \frac{-\Delta H^\circ}{RT} \right) \quad (12)$$

The assumptions of identical sites with no interactions between adsorbed molecules imply that the adsorption heat is independent on coverage. It follows by differentiation of Eq. (11) that the isosteric heat of sorption ( $-\Delta H^S$ ) is the same as the limiting heat of sorption ( $-\Delta H^\circ$ ):

$$\left( \frac{\partial \ln C}{\partial T} \right)_q = \frac{\Delta H^S}{RT^2} = \frac{d \ln K_L}{dT} = \frac{d \ln K'}{dT} = \frac{\Delta H^\circ}{RT^2} \quad (13)$$

To calculate it, both  $\Delta H^\circ$  and  $\Delta S^\circ$  can be first determined by the slope of the linear Van't Hoff plot, i.e. as  $\ln K_L$  versus  $(1/T)$  using equation:

$$\Delta H^\circ = \left[ R \frac{d \ln K}{d(1/T)} \right] \quad (14)$$

$\Delta H^\circ$  obtained here corresponds to isosteric heat of adsorption ( $\Delta H_{st,0}$ ) with zero surface coverage (i.e.  $q_e = 0$ ) [11].

Fig. 2 presents a Van't Hoff's plot of Langmuir isotherm, from which  $\Delta H^\circ = -64.96$  kJ/kg and  $\Delta S^\circ = -203.52$  J/kg/K have been obtained. If adsorption were a significant one, the change of free energy,  $\Delta G^\circ$ , should be negative. The negative value of  $\Delta S^\circ$  suggests decreased randomness at the solid/solution interface.

Using the Van't Hoff law, the  $K_L$  value versus temperature was determined. In order to complete this study, we assumed

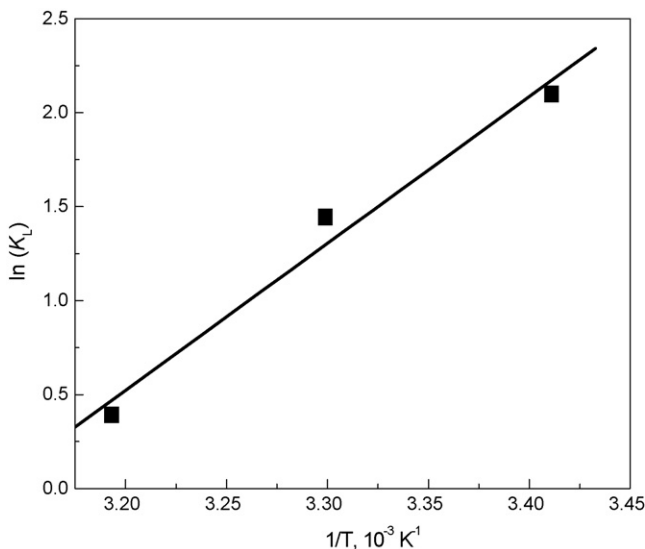


Fig. 2. Van't Hoff plot of adsorption equilibrium constant  $K$  using Langmuir isotherm.

to determine the  $q_m$  Langmuir parameter that represents the maximum capacity of active carbon at different temperatures. The evolution of this parameter is very important in the case of nonisothermal adsorption without assumptions.

According to the observed experimental point, we propose a linear function between temperature and the  $q_m$  value in the Langmuir model. The fitting model is

$$q_m = -T + 332.82 \quad (15)$$

with  $q_m$  (mg/g) and  $T$  (K).

As expected,  $q_m$  is decreased with an increase in temperature due to a decrease in adsorption capacities at higher temperatures. It can also be noticed that the difference in capacities at two different temperatures goes on increasing with an increase in concentration and reaches a constant value at a higher concentration level of surfactin. The decrease in saturation capacity with an increase in temperature can be explained on the basis of the fact that in the original Langmuir formulation the saturation limit was assumed to coincide with the saturation of a fixed number of identical surface sites and as such it should be independent on temperature. In fact, a modest decrease in saturation capacity with temperature is generally observed and is indeed to be expected provided that the saturation limit corresponds to filling of the micropore volume rather than with the saturation of a set of surface sites.

### 3.3. Adsorption of surfactin from culture media

A batch process was adopted to determine both the adsorption kinetics and adsorption capacity of surfactin from the culture media onto the activated carbon. Fig. 3 shows the kinetics of surfactin adsorption onto carbon with two different diameters of 0.9 and 1.4 mm at 37 °C. A decrease of activated carbon size was accompanied with an increase of adsorption rate. The adsorption capacities of surfactin from culture media onto the activated carbon for both diameters were 16.8 mg/g carbon, which was

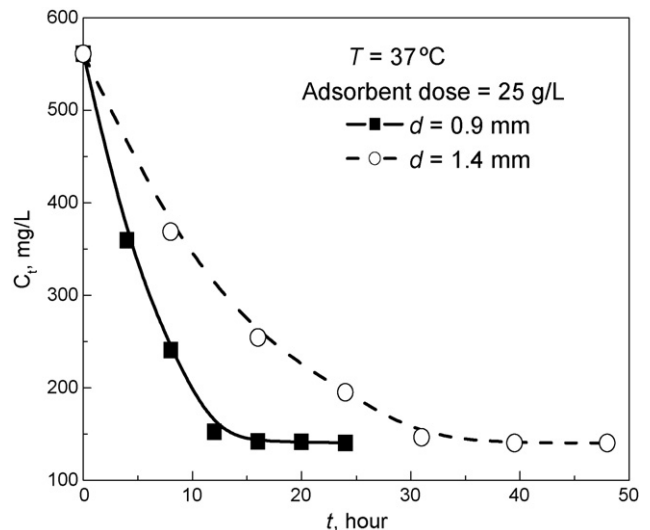


Fig. 3. Kinetics of surfactin adsorption from culture media onto activated carbons of two different diameters at 37 °C.

about 26% lower than that of pure surfactin from the aqueous solution, e.g. 22.8 mg/g carbon, calculated at 37 °C. The fact could be explained by the presence of other components in the media following the 48 hours fermentation. For example, glucose was also present while the strains' secretion contained other proteins. It is normal that the equilibrium concentration obtained in the solid was lower in the culture medium than the experimental points using the pure surfactin. The difference in adsorption capacity between single component solution and culture media is that the latter is a multicomponent system with significant interactive effects between solutes and adsorbent surface, and between solutes in solution. Also there are competitive effects between the solutes towards the adsorbent surface. In Fig. 3, the equilibrium concentration in the solid phase for the two different diameters, e.g. 1.4 and 0.9 mm, was similar, namely, 16.76 and 16.82 mg of surfactin per active carbon mass, respectively. To recover 90% of surfactin pure methanol was used.

#### 3.4. Kinetics modeling for surfactin adsorption from culture media

The simplest case to consider is a single microporous adsorbent particle exposed to a step change in sorbate concentration at external surface of the particle at time zero. Heat transfer is assumed to be sufficiently rapid, relative to the sorption rate, so that temperature gradients both the one through the particle and the one between particle and surrounding fluid are negligible.

Moreover, using the previous enthalpy value, the heat capacity of active carbon (0.93 J/(g K)) and the case which the adsorption capacity is the maximum in the temperature range 20–40 °C, the temperature in the active carbon pellet was increased to 2.8 °C with a adiabatic assumptions in the pellet (without heat transfer between the sold phase and the liquid).

The result shows that it is not necessary to take into account some nonisothermal adsorption conditions. In such case, we need to consider the temperature effect; a widely used model was developed by Ruthven et al. [12].

Heat conduction through adsorbent particles is generally much faster than heat transfer at the external surface and it is a sensible approximation to consider the particle as an essentially isothermal one with the heat transfer resistance being concentrated in the external film [13]. Thus, the energy equation for the solid can be represented in the following form:

$$-\Delta H \frac{d\bar{q}}{dt} = C_p \frac{dT}{dt} + h_s(T - T_0) \quad (16)$$

$$\text{With } \bar{q} = \frac{3}{r_c^3} \int_{r=0}^{r_c} r^2 q \cdot dr \quad (17)$$

For most particle shapes, the equivalent sphere is an acceptable approximation and the transport may therefore be described by a diffusion equation written in spherical coordinates:

$$\frac{\partial q}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 D_c \frac{\partial q}{\partial r} \right) \quad (18)$$

If diffusivity is constant, this equation simplifies to

$$\frac{\partial q}{\partial t} = D_c \left( \frac{\partial^2 q}{\partial r^2} + \frac{2}{r} \frac{\partial q}{\partial r} \right) \quad (19)$$

where  $D_c$  is intracrystalline diffusivity and  $q(r, t)$  is the adsorbed phase concentration. The initial and boundary conditions are:

$$q(r, 0) = 0, \quad \left( \frac{\partial q}{\partial r} \right)_{r=0} = 0$$

The equilibrium relationship at the solid surface is assumed to be linear because for surfactin adsorption, if the liquid concentration is higher than 5 mg/L, the maximum adsorption capacity depends just in temperature:

$$\frac{q'}{Q_e} = 1 + \left( \frac{\partial q^*}{\partial T} \right)_c \left( \frac{T - T_0}{Q_e} \right) \quad (20)$$

where  $q'$  is the adsorbed phase concentration at the solid surface and  $(\partial q^*/\partial T)_c$  is the slope equilibrium of the equilibrium line which is taken as constant over the step. The analytic solution is

$$\frac{\bar{q}(t)}{Q_e} = 1 - \sum_{n=1}^{\infty} \frac{9 \left[ \frac{(p_n \cot(p_n) - 1)}{p_n^2} \right]^2 \exp\left(-\frac{p_n^2 D_c t}{R_c^2}\right)}{\frac{1}{\beta} + \frac{3}{2} \left[ \frac{p_n \cot(p_n)(p_n \cot(p_n) - 1)}{p_n^2} + 1 \right]} \quad (21)$$

where  $p_n$  is given by the roots of the equation:

$$3\beta(p_n \cot(p_n) - 1) = p_n^2 - \alpha \quad (22)$$

and the parameter  $\alpha$  and  $\beta$  are defined by

$$\alpha = \frac{h_s R_c^2}{C_p D_c}, \quad \beta = \frac{\Delta H}{C_p} \left( \frac{\partial q^*}{\partial T} \right)_c$$

The corresponding expression for the temperature evolution is

$$\frac{T - T_0}{Q_e} \left( \frac{\partial q^*}{\partial T} \right)_c = \sum_{n=1}^{\infty} \frac{-3 \left[ (p_n \cot(p_n) - 1) / p_n^2 \right] \exp(-p_n^2 D_c t / R_c^2)}{(1/\beta) + (3/2) \left[ (p_n \cot(p_n)(p_n \cot(p_n) - 1) / p_n^2) + 1 \right]} \quad (23)$$

In the isothermal condition, it is necessary to use the Eq. (18) or (19). In this paper, the assumption is to consider a constant diffusivity value. The initial and boundary conditions are:

$$q(r, 0) = 0, \quad q(r_c, t) = Q_e, \quad \left( \frac{\partial q}{\partial r} \right)_{r=0} = 0$$

The analytic solution is

$$\bar{q}(t) = Q_e \left( 1 - \frac{6}{\pi^2} \sum \frac{1}{n^2} \exp\left(-\frac{\pi^2 n^2 D_c t}{R_c^2}\right) \right) \quad (24)$$

The  $D_c$  values by parameter identification (optimization method) are respectively  $7.86 \times 10^{-13}$  and  $7.5 \times 10^{-13}$  m<sup>2</sup>/s for  $R_c$  equal to 0.7 and 0.45 mm) (Fig. 4). In comparison to literature diffusion coefficient, the values obtained are similar.



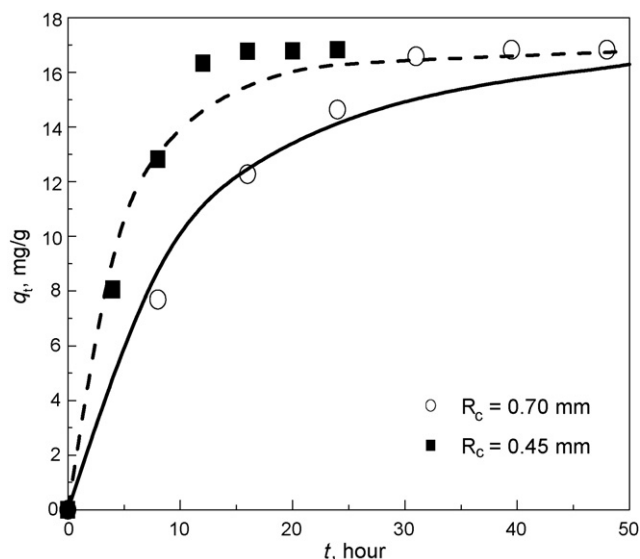


Fig. 4. Comparison between experiments (symbols) in Fig. 3 and modeling correlation (lines) for the kinetics of surfactin adsorption from culture media onto activated carbons using two different diameters at 37 °C.

For example, *n*-heptane/zeolite 5 Å at  $T = 298$  K, the diffusivity is  $3 \times 10^{-16}$  m<sup>2</sup>/s [14]. But compared to gas/solid adsorption, the value is very low; in general, the literature value was  $1 \times 10^{-6}$  m<sup>2</sup>/s.

#### 4. Conclusion

The thermodynamics of surfactin adsorption from aqueous solutions onto activated carbon has been studied. The Langmuir model is proposed to represent well the maximum adsorption capacity. The exothermic character of surfactin adsorption is demonstrated with  $\Delta H^\circ = -64.96$  kJ/kg and  $\Delta S^\circ = -203.52$  J/kg/K. The adsorption capacity from culture medium is found to be 26% lower than the pure one. Pellet diameter of 0.9 mm is proposed to ensure good recovery conditions. The modelling of surfactin adsorption on a single pellet

was realized. Based on the adsorption experiment, a correlation between the maximum adsorption capacity and temperature is proposed. Both adsorption equilibria and kinetics confirm that in real media, the activated carbon acts as an effective adsorbent for surfactin recovery.

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